PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 23795	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/14542	International filing date *(day/month/year)* 18.12.2003	Priority date *(day/month/year)* 18.12.2003
International Patent Classification (IPC) or both national classification and IPC INV. C12N15/62		
Applicant SEEGER, Werner et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20.06.2005	18.04.2006
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Bladier, C Telephone No. +49 89 2399-7306

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/14542

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-19 as originally filed

Sequence listings part of the description, Pages

1-39 as originally filed

Claims, Numbers

1-29 received on 06.02.2006 with letter of 31.01.2006

Drawings, Sheets

1/7-7/7 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

   see separate sheet

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☐ claims Nos.

   because:

   ☒ the said international application, or the said claims Nos. 27-29 relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-29

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-29
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-29
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-26
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item I
Basis of the report

1. The present report is based on the amended sequence listing which has been filed on 18.03.2004 and which comprises the sequences as identified by SEQ ID N°1 to 26. This amended sequence listing complies with the requirement of Article 34(2)(b) PCT.

2. An amended set of **claims 1-29** has been filed with letter dated 31.01.2006.

   a) amended **claims 12** and **13** comply with the requirement of Article 34(2)(b) PCT.

   b) **claim 11** has been amended by deleting the non mammalian plasminogen activators "desmodus salivary plasminogen activator alpha-1", "streptokinase2, and "staphylokinase". This amendment complies with the requirement of Article 34(2)(b) PCT.

   c) **claim 11** has also been amended by replacing the wording "and catalytically active mutants thereof" with the wording "and catalytically active mutants of the plasminogen activator". This amendment broadens the subject-matter of claim 11 since it now encompasses mutants of any plasminogen activator, *i.e.* mutants of the plasminogen activators of claim 11 and mutants of any other type of plasminogen activator. No basis can be found in the description or in the claims as originally filed for said broadening. Therefore, this amendment contravenes the provisions of Article 34(2)(b) PCT. This report has thus been established as if this amendment had not been made (Rule 70.2(c) PCT).

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

3. An incomplete ISR has been established for claims 1-29 due to the fact that their subject-matter is not sufficiently disclosed and supported (Articles 5 and 6 PCT) (see PCT/ISA/210). Accordingly the present written report is only performed on parts of **claims 1-29** that have been searched, *i.e.* on parts of claims 1-29 relating to a fusion

protein comprising (a) a mammalian pulmonary surfactant protein (b) a mammalian plasminogen activator selected from the group consisting of HMW-u-PA, LMW-u-PA, LMW-u-PA B-chain, r-scu-PA, t-PA, rt-PA (Rule 66.1(e) PCT).

4. **Claims 27-29** relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Cited documents

5. Reference is made to the following document:

D1: RUPPERT C. *et al.*, 'Chemical crosslinking of urokinase to pulmonary surfactant protein B for targeting alveolar fibrin' THROMBOSIS AND HAEMOSTASIS, vol. 89, 2003, p53-64

Novelty

6. The subject-matter of **claims 1-29**, limited as indicated under Item III-3 above, is not revealed in the available prior art. Consequently, these claims are novel according to Article 33(2) PCT.

Inventive step

7. **Claims 1-29**, limited as indicated under Item III-3 above, meet the requirement of Article 33(3) PCT with respect to inventive step for the following reason:

Document D1, which is considered to represent the closest state of the art, relates to an hybrid molecule obtained by chemical cross-linking of a mammalian pulmonary surfactant (the mature surfactant protein SP-B) and a mammalian plasminogen

activator (the B-chain of urokinase plasminogen activator).

The subject-matter of the present application differs from the subject-matter of document D1 in that the mammalian pulmonary surfactant is fused to the mammalian plasminogen activator instead of being chemically cross-linked.

According to the description (see p4 l23 to p5 l2), the effect associated to this difference is that the fusion protein can be obtained in an easier way and in amount sufficient for therapeutic applications via recombinant production.

Starting from this point, the problem which is derived is how to obtain an hybrid protein consisting of a mammalian pulmonary surfactant and a mammalian plasminogen activator in an easier way and in amount sufficient for therapeutic applications.

The solution provided by the application is to obtain the hybrid protein by recombinant production of a protein consisting of either a mature mammalian pulmonary surfactant or a mammalian pulmonary surfactant precursor lacking its C-terminal propeptide fused with a mammalian plasminogen activator.

In view of the above-defined problem, the question to be answered for the evaluation of the inventive step is whether this solution was obvious to a person skilled in the art.

The use of recombinant fusion protein to circumvent problems inherent in chemical coupling was common general knowledge at the time of the application. However the IPEA is of the opinion that the skilled person would not have been motivated to generate a fusion protein of the mature pulmonary surfactant with a mammalian plasminogen activator. Actually, no evidence of the recombinant production of the mature mammalian pulmonary surfactant is present in the available state of the art. According to the present application (cf p5 l31-35), this is due to the hydrophobicity of the mature mammalian pulmonary surfactant that disrupts lipid membranes which results in cell lysis. The skilled person would also not have been motivated to use a mammalian pulmonary surfactant protein precursor lacking its C-terminal propeptide in a fusion protein with a mammalian plasminogen activator since the surfactant protein precursor is not expected to exhibit the biophysical activity of the mature

surfactant protein. Consequently the IPEA is of the opinion that replacing the chemically cross-linked conjugate of D1 with the recombinant production of a protein consisting of either a mature mammalian pulmonary surfactant or a mammalian pulmonary surfactant protein precursor lacking its C-terminal propeptide fused to a mammalian plasminogen activator in order to produce the hybrid protein in an easier way and in amount sufficient for therapeutic applications involves an inventive step in the sense of Article 33(3) PCT.

Industrial application - Article 33(4) PCT

7. For the assessment of the present **claims 27-29** on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Certain observations on the international application

8. The term "mutants" renders the scope of claim 11 obscure since it is unclear which mutations are made and to which extent (structurally and functionally) said mutants differ from the mammalian plasminogen. Furthermore the wordings "catalytically active" does not characterise any further said mutants since it is not clear to which function it is referred to. Consequently **claim 11** lacks clarity (Article 6 PCT).

New Claims

1. A fusion protein comprising:
(a) a mammalian surfactant protein precursor lacking its C-terminal propeptide, and
(b) a mammalian plasminogen activator,
wherein the surfactant protein precursor is fused at its C-terminus to the N-terminus of the plasminogen activator.

2. The fusion protein of claim 1, wherein one of the protein components (a) or (b) is a human protein.

3. The fusion protein of claim 1 or 2, wherein both protein components (a) and (b) are human proteins.

4. The fusion protein of any of claims 1 to 3, wherein the surfactant protein precursor is selected from surfactant protein B (SP-B) or surfactant protein C (SP-C).

5. The fusion protein of any of claims 1 to 4, wherein the surfactant protein precursor is surfactant protein B (SP-B).

6. A fusion protein comprising:
(a) a mature mammalian surfactant protein, and
(b) a mammalian plasminogen activator,
wherein the mature surfactant protein is fused at its C-terminus or its N-terminus to the N-terminus or the C-terminus of the plasminogen activator, respectively.

7. The fusion protein of claim 6, wherein one of the protein components (a) or (b) is a human protein.

8. The fusion protein of claim 6 or 7, wherein both protein components (a) and (b) are human proteins.

9. The fusion protein of any of claims 6 to 8, wherein the mature surfactant protein is selected from the group consisting of surfactant protein B (SP-B), and surfactant protein C (SP-C).

10. The fusion protein of any of claims 6 to 9, wherein the mature surfactant protein is surfactant protein B (SP-B).

11. A fusion protein of any of claims 1 to 10, wherein the mammalian plasminogen activator is selected from the group consisting of high molecular weight two-chain urokinase-plasminogen activator (HMW-u-PA), low molecular weight two-chain u-PA (LMW-u-PA), low molecular weight u-PA B-chain, recombinant single-chain u-PA (r-scu-PA), tissue-plasminogen activator (t-PA), recombinant t-PA (rt-PA), its variants r-PA, n-PA, and TNK-t-PA, and catalytically active mutants of the plasminogen activator.

12. The fusion protein according to any of claims 1 to 5 comprising the surfactant protein B (SP-B) precursor N-terminally fused to the low molecular weight two-chain u-PA (LMW-u-PA), as shown in SEQ ID NO: 19 and SEQ ID NO: 20, respectively.

13. The fusion protein according to any of claims 6 to 10 comprising the mature surfactant protein B (SP-B) fused to the low molecular weight two-chain u-PA (LMW-u-PA), as shown in SEQ ID NO: 25 and SEQ ID NO: 26, respectively.

14. The fusion protein of any of claims 1 to 13, which carries a protein or peptide affinity tag at its N-terminus and/or at its C-terminus.

15. A nucleic acid molecule comprising a nucleotide sequence encoding a fusion protein of any of claims 1 to 14.

16. The nucleic acid molecule comprising the nucleotide sequence of SEQ ID No: 6 or SEQ ID NO: 7.

17. The nucleic acid molecule comprising the nucleotide sequence of SEQ ID No: 12 or SEQ ID NO: 13.

18. The nucleic acid molecule according to any of claims 15 to 17, wherein the nucleic acid molecule is operably linked to a regulatory sequence to allow expression of the nucleic acid molecule.

19. The nucleic acid molecule according to claim 18, wherein the regulatory sequence comprises a promoter sequence and a transcription termination sequence.

20. The nucleic acid molecule of any of claims 15 to 19 comprised in a vector.

21. A host cell containing a nucleic acid molecule of any of claims 15 to 20.

22. A method for production of a fusion protein of any of claims 1 to 14, comprising:
(a) introducing a nucleic acid molecule encoding the fusion protein into a suitable vector, and
(b) introducing the recombinant vector obtained in (a) into a suitable host cell or into a suitable cell extract.

23. A pharmaceutical composition comprising a fusion protein of any of claims 1 to 14.

24. Use of a fusion protein of any of claims 1 to 14 for the manufacture of a pharmaceutical composition.

25. The use of claim 24, wherein the pharmaceutical composition is for prevention and/or treatment of inflammatory and interstitial lung diseases.

26. The use of claim 24 or 25, wherein the pharmaceutical composition has fibrinolytic activity.

27. A method of prevention and/or treatment of inflammatory and interstitial lung diseases, comprising the step of administering a fusion protein of any of claims 1 to 14 to a mammal at a dose sufficient to prevent and/or treat the disease.

28. The method according to claim 27, wherein the fusion protein is administered to a mammal by an administration selected from the group consisting of parenteral administration, non-parenteral (enteral) administration, and topical administration.

29. The method according to claim 28, wherein parenteral administration is by aerosol administration or intratracheal instillation.